Life Sciences
Volume 61, Issue 4 , 20 June 1997, Pages 355-361

doi:10.1016/S0024-3205(97)00406-2 Cite or Link Using DOI

Effects of clobenpropit (VUF-9153), a histamine H_3-receptor antagonist, on learning and memory, and on cholinergic and monoaminergic systems in mice

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Revised 21 April 1997. Available online 16 December 1997.

Abstract

The effects of clobenpropit (VUF-9153), a potent histamine H_3-receptor antagonist, on a scopolamine-induced learning deficit in the step-through passive avoidance test was studied in mice. Clobenpropit (10 and 20 *mg/kg*) alone showed a tendency to ameliorate the scopolamine-induced learning deficit, and clobenpropit (10 *mg/kg*) in combination with zolantidine (20 *mg/kg*), a histamine H_2-receptor antagonist, ameliorated the scopolamine-induced effect. This ameliorating effect was antagonized by (R)-α-methylhistamine (20 *mg/kg*), a histamine H_3-receptor agonist and pyrilamine (20 *mg/kg*), a histamine H_1-receptor antagonist, suggesting that clobenpropit in combination with zolantidine showed the ameliorating effect *via* histamine H_3 receptors and/or histamine H_1 receptors. We also studied the effects of clobenpropit on cholinergic and monoaminergic systems. Clobenpropit did not show any significant effect on these neuronal systems except the activation of noradrenergic system. The present results suggest that the effect of clobenpropit might be

partially involved with the activation of noradrenergic system, and the histaminergic system may play certain important roles in learning and memory.

Author Keywords: histamine; histamine H_3 receptor; clobenpropit; learning; memory; passive avoidance test

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Life Sciences
Volume 61, Issue 4 , 20 June 1997, Pages 355-361

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ACCESSION NUMBER: **1970:433587** CAPLUS
DOCUMENT NUMBER: 73:33587
TITLE: Potential antiarrhythmics related to 2-phenylethanol diethylaminoethyl ether. II. Pharmacological study
AUTHOR(S): Molimard, Robert; Morin, Robert; Eskenazi, Pierre; Motosso, Francoise; Vigier, Denise
CORPORATE SOURCE: Lab. Jacques Logeais, Issy-les-Moulineaux, Fr.
SOURCE: Chimica Therapeutica (1970), 5(1), 10-15
CODEN: CHTPBA; ISSN: 0009-4374
DOCUMENT TYPE: Journal
LANGUAGE: French

AB The antiarrhythmic properties of numerous phenylalkoxy alkyl amines were compared on isolated rabbit hearts (lengthening of refractory period) and in rabbits, mice, and rats (protection against aconitine- and CHCl3-induced fibrillation and against arrhythmia due to coronary ligation). Among the phenylethoxyethylamines, those rings substituted with H, Cl, or Me, such as dimethyl[2-(2-phenylethoxy)ethyl]amine and) trip-tolylethoxyethyl]amine, were most active; however, these compds. were generally neurotoxic. Compds. with unsubstituted amine groups, such as p-chlorophenylethoxyethylamine, were less active and less neurotoxic. Antiarrhythmic activity and neurotoxicity increased with the length of the alkoxy chain, as exemplified by 2-(3-diethylphenylpropoxy)triethylamine and 2-(4-phenylbutoxyethylamine.

IT **27078-39-3**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(antiarrhythmic action of)

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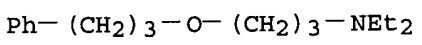

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TITLE: Potential antiarrythmics related to 2-phenylethanol diethylaminoethyl ether. I. Synthesis
AUTHOR(S): Maillard, Jacques; Langlois, Michel; Meingan, Jean P.; Remond, Georges; Jolly, Raymond
CORPORATE SOURCE: Lab. Jacques Logealis, Issay-les-Moulineaux, Fr.
SOURCE: Chimica Therapeutica (1970), 5(1), 1-9
CODEN: CHTPBA; ISSN: 0009-4374
DOCUMENT TYPE: Journal
LANGUAGE: French
GI For diagram(s), see printed CA Issue.
AB Antiarrythmic aminoalkyl phenylalkyl ethers (I), where n is 2 or 3, R is alkyl or (R2N =) piperidino; R1 is alkylene, substituted alkylene, or OCH2CH2; and R3 and (or) R4 is H, Cl, Me, alkoxy, CF3, or NH2, were prepared by treating a 1-dialkylamino-2-chloroalkane with a substituted 2-phenylalkanol Na derivs. I may be converted into its nitro and amino derivs.
IT **27078-39-3P**
RL: SPN (Synthetic preparation); PREP (Preparation)
(preparation of)
RN 27078-39-3 CAPLUS
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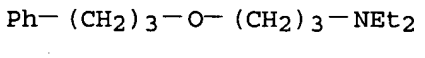

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TITLE: Effects of clobenpropit (VUF-9153), a histamine H3-receptor antagonist, on learning and memory, and on cholinergic and monoaminergic systems in mice
AUTHOR(S): Miyazaki, Shuichi; Onodera, Kenji; Imaizumi, Masahiro; Timmerman, Hendrik
CORPORATE SOURCE: Biology Laboratory, Research & Development Division, Yamasa Corporation, Chiba, 288, Japan
SOURCE: Life Sciences (1997), 61(4), 355-361
CODEN: LIFSAK; ISSN: 0024-3205
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The effects of clobenpropit on a scopolamine-induced learning deficit in the step-through passive avoidance test was studied in mice. Clobenpropit (10 and 20 mg/kg) tended to antagonize the scopolamine-induced learning deficit, and clobenpropit (10 mg/kg) in combination with zolantidine (20 mg/kg), a histamine H2-receptor antagonist, further antagonized the scopolamine-induced effect. This ameliorating effect was antagonized by (R)-α-methylhistamine (20 mg/kg), a histamine H3-receptor agonist, and pyrilamine (20 mg/kg), a histamine H1-receptor antagonist, suggesting that clobenpropit in combination with zolantidine produces an ameliorating effect via histamine H3 receptors and/or histamine H1 receptors. The effects of clobenpropit on cholinergic and monoaminergic systems were also studied. Clobenpropit did not produce any significant effect on these neuronal systems except the activation of the noradrenergic system. The results suggest that the effect of clobenpropit might be partially via activation of the noradrenergic system, and the histaminergic system may play certain important roles in learning and memory.
IT **104076-38-2**, Zolantidine
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(clobenpropit effect on learning and memory stimulation by)
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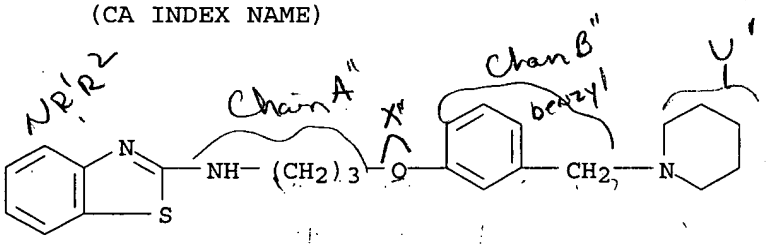

Life Sciences, Vol. 61, No. 4, pp. 355-361, 1997

0024-3205/97 $17.00 + .00

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PII S0024-3205(97)00406-2

EFFECTS OF CLOBENPROPIT (VUF-9153), A HISTAMINE H_3-RECEPTOR ANTAGONIST, ON LEARNING AND MEMORY, AND ON CHOLINERGIC AND MONOAMINERGIC SYSTEMS IN MICE

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(Received in final form April 21, 1997)

Summary

The effects of clobenpropit (VUF-9153), a potent histamine H_3-receptor antagonist, on a scopolamine-induced learning deficit in the step-through passive avoidance test was studied in mice. Clobenpropit (10 and 20 mg/kg) alone showed a tendency to ameliorate the scopolamine-induced learning deficit, and clobenpropit (10 mg/kg) in combination with zolantidine (20 mg/kg), a histamine H_2-receptor antagonist, ameliorated the scopolamine-induced effect. This ameliorating effect was antagonized by *(R)*-α-methylhistamine (20 mg/kg), a histamine H_3-receptor agonist and pyrilamine (20 mg/kg), a histamine H_1-receptor antagonist, suggesting that clobenpropit in combination with zolantidine showed the ameliorating effect *via* histamine H_3 receptors and/or histamine H_1 receptors. We also studied the effects of clobenpropit on cholinergic and monoaminergic systems. Clobenpropit did not show any significant effect on these neuronal systems except the activation of noradrenergic system. The present results suggest that the effect of clobenpropit might be partially involved with the activation of noradrenergic system, and the histaminergic system may play certain important roles in learning and memory.

Key Words: histamine, histamine H_3 receptor, clobenpropit, learning, memory, passive avoidance test

In addition to postsynaptic histamine H_1 and H_2 receptors in the brain, the existence of presynaptic histamine H_3 receptors which control the release of neuronal histamine has been suggested (1, 2). Histamine H_3-receptor antagonists, such as thioperamide and GT-2016 can activate the central histaminergic system inducing enhanced histamine release from nerve terminals (3, 4). Since its development by Van der Goot et al. clobenpropit (VUF-9153) has been known as a potent and selective histamine H_3-receptor antagonist comparable to thioperamide *in vitro* (5-8). For example, clobenpropit is approximately 10-times and 6-times more potent than thioperamide in its affinity for histamine H_3 receptors and in its activity of increasing histamine release, respectively. We

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showed that the anticonvulsant effect of clobenpropit was approximately 10-times more potent than that of thioperamide in mice (9).

The central histaminergic system is thought to play important roles in learning and memory in rodents. For example, activation of the histaminergic system by intracerebroventricular treatment with histamine or intraperitoneal administration of histidine leads to improved learning and memory in rodents (10-12). Inhibition of the central histaminergic system by blocking H_1 receptors or histamine synthesis, disrupts learning and memory (11). Betahistine, a partial H_1-receptor agonist and H_3-receptor antagonist improved a scopolamine-induced learning deficit in rat (13). Previously, we also reported that thioperamide in combination with zolantidine ameliorated a scopolamine-induced learning deficit in mice, presumably by a partial contribution of the central cholinergic system (14, 15).

Histamine H_3-receptors have been reported to regulate not only the release and turnover of histamine *via* autoreceptors on histaminergic nerve endings (3, 16), but also the release of noradrenaline, dopamine, serotonin, and acetylcholine *via* heteroreceptors on non-histaminergic axon terminals (17). Thioperamide reportedly increased the release of these neurotransmitters, while histamine and *(R)*-α-methylhistamine, a histamine H_3-receptor agonist, decreased them *via* histamine H_3 heteroreceptors *in vitro* (18-21).

In this study, we examined the effect of clobenpropit on a scopolamine-induced learning deficit in a passive avoidance test to confirm the involvement of the histaminergic system in learning and memory. Furthermore, we investigated the contribution of other neuronal systems to the effects of clobenpropit by measuring the content of acetylcholine, monoamines, and their metabolites in brain regions of mice.

Materials and Methods

Animals

Male ICR mice (Clea Japan, Inc., Tokyo, Japan), aged 6 weeks and weighing 30-35 g, were used. The animals were housed under standard conditions (23 ± 1 °C, light-dark cycle with the light on from 7:00 to 19:00) with free access to water and food in their home cages. Experiments were performed between 13:00 and 17:00.

Drugs

All drugs were administered i.p. in a volume of 10 ml/kg. Clobenpropit dihydrobromide was synthesized by Van der Goot (Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands). Scopolamine hydrobromide (Wako Pure Chemical Industries, Ltd., Osaka, Japan), pyrilamine maleate, *(R)*-α-methylhistamine dihydrochloride (Research Biochemicals Inc., Natick, MA, USA), zolantidine dimaleate (a gift from Smith-Kline & Beecham Laboratories Inc., London, UK), and clobenpropit were dissolved in physiological saline. Scopolamine, clobenpropit, and zolantidine were injected 15, 60, and 70 min, respectively, before an acquisition trial on day 1 in a behavioral study. In tests of the antagonism of the effect of clobenpropit plus zolantidine, *(R)*-α-methylhistamine or pyrilamine was injected 80 min before the acquisition trial. Clobenpropit was injected 60 min before microwave irradiation in the biochemical study.

Step-through passive avoidance test

The step-through passive avoidance test was performed as described previously (15). Briefly, an acquisition trial was performed as follows: the mice were placed in a light compartment facing away from a dark compartment. When the mice entered the dark compartment, an electrical foot shock (constant voltage: 75 V) was delivered to the grid. Twenty-four hours later, a retention trial was performed in the same manner as an acquisition trial, and the latency for entering the dark compartment (step-through latency) was recorded. If the mice did not enter the dark compartment within 300 sec in the retention trial, the test was stopped and the step-through latency was recorded as 300 sec.

Measurement of brain neurotransmitters

Tissue preparation

The mice were sacrificed by microwave irradiation (4 kW, 1.2 s) using a microwave applicator (TMW-6402, Toshiba, Tokyo, Japan). Their brains were quickly removed and dissected into the following four regions: cerebral cortex, diencephalon, midbrain, and pons and medulla oblongata as described previously (22). These samples were weighed and added 200 ng of isoproterenol (Research Biochemicals Inc.) and 5 nmol of ethylhomocholine (Eicom Co., Kyoto, Japan), as internal standards for monoamine and acetylcholine, respectively. These materials were homogenized with ultrasonic generator (Model US-150T; Nihonseiki Co., Ltd., Tokyo, Japan) in 0.2 M perchloric acid containing 0.1 mM ethylenediamine tetraacetic acid disodium (Na_2EDTA). The homogenate was adjusted to pH 3.0 by adding 1 M CH_3COONa, and was then centrifuged at 18,000 g for 15 min at 4 °C. The supernatant was filtered through a 0.45 μm membrane filter and used as an assay sample.

Chromatographic conditions

Acetylcholine and choline were analyzed by the method as described previously (15). The HPLC system (Hitachi Co., Ltd., Tokyo, Japan) consisted of a guard column (Eicom Prepak Set), an analytical column (Eicompak AC-GEL, 150 mm × 6.0 mm i.d.), an immobilized enzyme column (Eicom AC-Enzympak), and a catecholamine trap column (Eicom CA-Trap, all columns from Eicom) at 33 °C. Acetylcholine and choline were detected using an electrochemical detector (a model ECD-100) with a platinum electrode (WE-PT, these from Eicom) with the 450-mV detector potential. The mobile phase consisted of 0.1 M $Na_2HPO_4 \cdot 12H_2O$ containing 200 mg sodium decane sulfonate and 65 mg tetramethylammonium chloride per liter, adjusting to pH 8.5 by H_3PO_4. The flow rate was 1 ml/min.

Monoamines and their metabolites: noradrenaline, 3-methoxy-4-hydroxy-phenylethylene glycol (MHPG), dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanilic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were separated using an HPLC system (Hitachi) as described previously (23). Briefly, the samples were injected into the HPLC system, which consist of a guard column (Eicom Prepak Set) and an analytical column (Eicompak MA 5-ODS, 150 mm × 4.6 mm i.d., both columns from Eicom) at room temperature. Monoamines and their metabolites were detected using the ECD with a graphite electrode (WE-3G, Eicom) with the 750-mV detector potential. The mobile phase consisted of 0.1 M sodium acetate-citric acid buffer (9:10) containing 200 mg sodium 1-octane sulfonate, 5 mg Na_2EDTA, and 150 ml of methanol per liter. The flow rate was 1 ml/min.

Statistical analysis

We used the step-through latency (sec) on the retention trial as an index of learning effects, with results expressed as the mean ± standard error (S.E.). Statistical significance was determined by analysis of variance (ANOVA) followed by the Mann-Whitney *U*-test (2-tailed) in the behavioral study. Biochemical data were converted to percentages relative to the vehicle-treated control and expressed as the mean ± S.E., and statistical significance was determined by ANOVA followed by the Dunnett *t*-test (2-tailed).

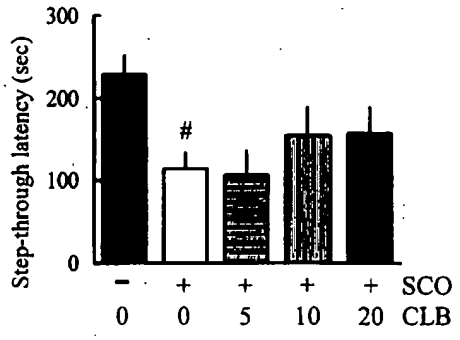

Fig. 1

Effect of clobenpropit on scopolamine-induced shortening of the step-through latency in the passive avoidance test. Clobenpropit (CLB; 5, 10, and 20 mg/kg) and scopolamine (SCO; 1 mg/kg) were administered i.p. 60 and 15 min, respectively, before the acquisition trial. Physiological saline was injected into the reference group. Each column and bar represents the step-through latency in the retention trial as the mean ± S.E. of 10 mice. Significant difference: # $p < 0.05$ versus the vehicle-treated control group.

Results

Effects of clobenpropit on the scopolamine-induced learning deficit

Treatment with scopolamine (1 mg/kg, i.p.) significantly shortened step-through latency in the retention trial compared with the vehicle-treated control group in all cases. Clobenpropit (10 and 20 mg/kg, i.p.) alone showed a tendency to reverse the scopolamine-induced shortening of step-through latency in the retention trial (Fig. 1). Clobenpropit alone or in combination with zolantidine did not affect the step-through latency at the dose tested in the retention trial compared with the vehicle-treated control group (data not shown). Clobenpropit (10 mg/kg, i.p.) in combination with zolantidine (20 mg/kg, i.p.) significantly improved the scopolamine-induced shortening of step-through latency in the retention trial, and this ameliorating effect was antagonized by *(R)*-α-methylhistamine (20 mg/kg, i.p., Fig. 2A) and pyrilamine (20 mg/kg, i.p., Fig. 2B). *(R)*-α-Methylhistamine or pyrilamine alone did not affect the step-through latency at the dose tested in the retention trial compared with the vehicle-treated control group. Zolantidine (20 mg/kg) alone affected neither the step-through latency in the retention trial nor the scopolamine-induced shortening of the step-through latency (data not shown).

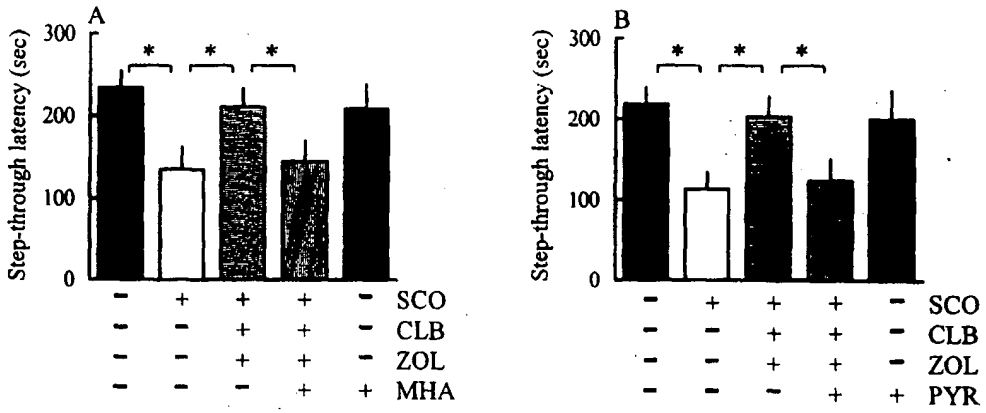

Fig. 2

Effects of clobenpropit plus zolantidine on scopolamine-induced shortening of the step-through latency, and the antagonism of *(R)*-α-methylhistamine (A) and pyrilamine (B) in the passive avoidance test. *(R)*-α-methylhistamine (MHA; 20 mg/kg) or pyrilamine (PYR; 20 mg/kg), zolantidine (ZOL; 20 mg/kg), clobenpropit (CLB; 10 mg/kg), and scopolamine (SCO; 1 mg/kg) were administered i.p. 80, 70, 60, and 15 min, respectively, before the acquisition trial. Physiological saline was injected into the reference groups. Each column and bar represents the step-through latency in the retention trial as the mean ± S.E. of 15 mice. Significant difference: * $p < 0.05$.

Effects of clobenpropit on brain neurotransmitters

Clobenpropit alone or in combination with zolantidine did not affect acetylcholine or choline levels (data not shown), (HVA+DOPAC)/dopamine ratio, or 5-HIAA/5-HT ratio (Figs. 3B, C), at doses tested in any regions examined. Clobenpropit (10 and 20 mg/kg) alone or in combination with zolantidine significantly increased MHPG/noradrenaline ratio in midbrain and/or pons and medulla oblongata (Fig. 3A).

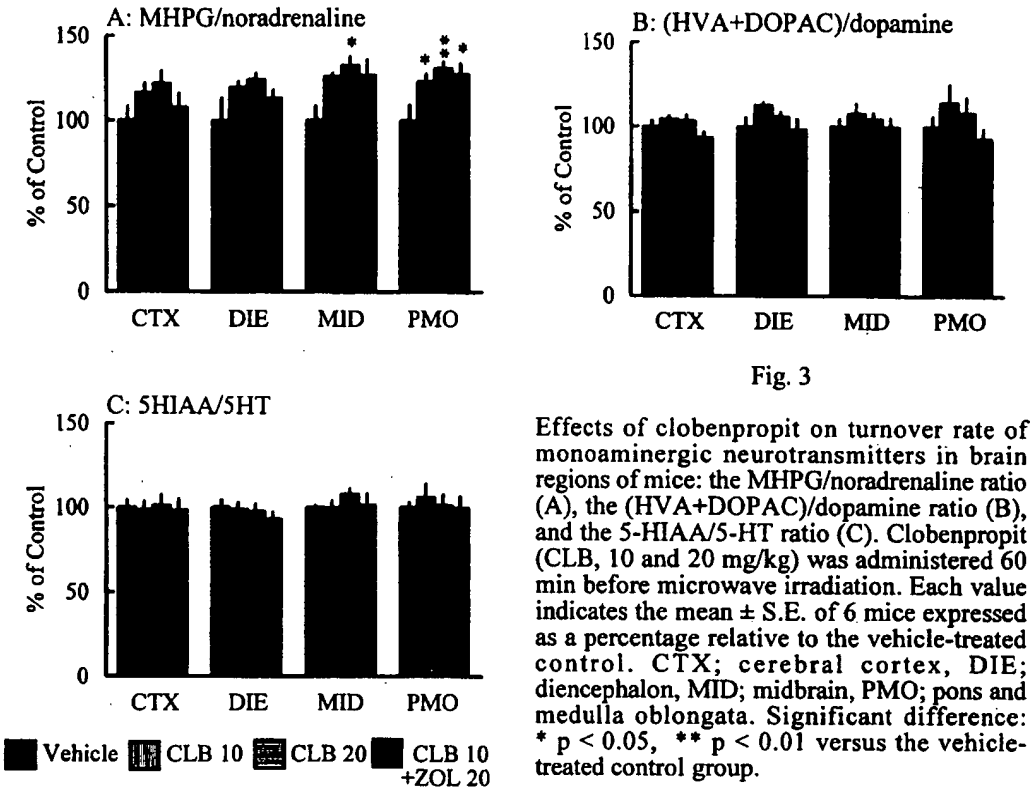

Fig. 3

Effects of clobenpropit on turnover rate of monoaminergic neurotransmitters in brain regions of mice: the MHPG/noradrenaline ratio (A), the (HVA+DOPAC)/dopamine ratio (B), and the 5-HIAA/5-HT ratio (C). Clobenpropit (CLB, 10 and 20 mg/kg) was administered 60 min before microwave irradiation. Each value indicates the mean ± S.E. of 6 mice expressed as a percentage relative to the vehicle-treated control. CTX; cerebral cortex, DIE; diencephalon, MID; midbrain, PMO; pons and medulla oblongata. Significant difference: * $p < 0.05$, ** $p < 0.01$ versus the vehicle-treated control group.

Discussion

Clobenpropit in combination with zolantidine significantly improved the learning deficit produced by scopolamine. This is consistent with our previous finding that thioperamide plus zolantidine ameliorated a scopolamine-induced learning deficit using the same apparatus (15) and the elevated plus-maze test (14) in mice. These data show that the potency of clobenpropit against a scopolamine-induced learning deficit is approximately 2-fold higher than that of thioperamide, while we previously found that clobenpropit was approximately 10 times more potent than thioperamide against electrically-induced convulsions (9). Since the ameliorating effect of clobenpropit in combination with zolantidine was antagonized by *(R)*-α-methylhistamine, a histamine H_3-receptor agonist, the ameliorating effect of clobenpropit and zolantidine is probably due to the increased release of endogenous histamine *via* autoreceptors on histaminergic neurons. This is supported by our previous data in which clobenpropit dose-dependently decreased histamine levels and increased histidine decarboxylase activity in the mouse brain, and the blockage of histamine H_3 receptors leads to enhance neuronal histamine release, resulting in lower histamine levels in tissue homogenates (9, 24). Moreover, the ameliorating effect was antagonized by pretreatment with pyrilamine, a histamine H_1-receptor antagonist, in this study. This result suggested that the ameliorating effect of clobenpropit plus zolantidine is also mediated through postsynaptic histamine H_1 receptors. However, it is notable that clobenpropit or thioperamide alone could not significantly improve the scopolamine-induced learning deficit. Clobenpropit blocks histamine H_3 receptors, and then increases neuronal histamine, which in turn stimulates both postsynaptic histamine H_1 and H_2 receptors. 2-Methylhistamine, a histamine H_1-receptor agonist, facilitates memory, and 4-methylhistamine, a histamine H_2-receptor agonist, worsens memory (25). Therefore, it is conceivable that stimulation of histamine H_1 receptors may improve the learning deficit, but stimulation of histamine H_2 receptors may have the opposite effect.

On the other hand, the central cholinergic system is also known to play an important role in learning and memory (26, 27), and there is a close relationship between the cholinergic and the histaminergic systems. For example, the memory facilitating effect of 2-methylhistamine was attenuated by a muscarinic antagonist, pirenzepine (25). Conversely, activation of the central histaminergic system could antagonize the scopolamine-induced learning deficit, as shown here and in previous studies (12-15). Thioperamide-induced increase of acetylcholine release was mediated by histamine H_3 heteroreceptors *in vitro* and *in vivo* (19, 28). Therefore, we considered the possibility that clobenpropit, besides acting on the histaminergic system, acted *via* the cholinergic system in producing its ameliorating effect. However, we could not detect any changes in acetylcholine or choline levels, probably because histamine H_3 heteroreceptors played a minor role in the modulation of acetylcholine release. Previous and present data suggest that the contribution of the cholinergic system in the ameliorating effect induced by histamine H_3-receptor antagonists is not mediated by histamine H_3 heteroreceptors, but by postsynaptic histamine H_1 receptors. This is supported by previous reports that histamine excites cholinergic neurons through histamine H_1 receptors (29, 30).

Within the monoaminergic systems we observed that clobenpropit increased turnover rate of noradrenaline only in some brain regions, although histamine H_3 heteroreceptors reportedly modulate the release of noradrenaline, dopamine, and serotonin (18, 20, 21). Thus, it appears that the contribution of histamine H_3 heteroreceptors on the modulation of monoaminergic neurotransmitters may be minor, just as with the cholinergic system. On the other hand, we previously observed that histidine, a histamine precursor, also increased turnover rate of noradrenaline (31). Intracerebroventricular administration of histamine activated noradrenergic neurons *via* histamine H_1 receptors (32). Taking together the present data and previous reports, we conclude that the activation of the histaminergic system may lead to stimulation of the noradrenergic system *via* histamine H_1 receptors. Compton et al. reported that bilateral lesions of the locus coeruleus, a noradrenergic nucleus, impaired learning and memory (33). In the present study the locus coeruleus was located within the pons and medulla oblongata. Therefore, the clobenpropit effect may partially be mediated by the noradrenergic system. On the other hand, the effect of clobenpropit on noradrenergic metabolism was not affected by pretreatment with zolantidine, although clobenpropit plus zolantidine ameliorated the scopolamine-induced learning deficit. Histamine H_2-receptors may modulate learning and memory by other mechanism(s) in mice.

Finally, the present results strengthen the idea that the histaminergic system may play important roles in learning and memory. However, much more evidence is needed to discuss the function of histamine H_3 receptors, especially on heteroreceptors.

Acknowledgments

We thank for supplying zolantidine Smith-Kline & Beecham Laboratories., Inc. (London, UK). We thank Dr. H. Machida, Yamasa Corporation, for his support and reviewing the manuscript.

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